

CLAIMS

We claim:

1. An isolated nucleic acid molecule consisting essentially of a nucleotide sequence that encodes a microbial β -glucuronidase, provided that the microbial β -glucuronidase is not *E. coli* β -glucuronidase.

2. The nucleic acid molecule of claim 1, wherein the microbial β -glucuronidase is encoded by a nucleic acid molecule comprising nucleotides 1-1689 of Figures 4I-J or by a nucleic acid molecule that hybridizes under stringent conditions to the complement of nucleotides 1-1689 of Figure 4I-J and which encodes a functional β -glucuronidase.

3. The nucleic acid molecule of claim 1, wherein the microbial β -glucuronidase comprises the amino acid sequences of Figure 5B, or a variants thereof, and which encodes a functional β -glucuronidase.

4. The nucleic acid molecule of claim 1, wherein the microbe is a eubacteria.

5. The nucleic acid molecule of claim 4, wherein the eubacteria is selected from the group consisting of purple bacteria, gram(+) bacteria, cyanobacteria, spirochaetes, green sulphur bacteria, bacteroides and flavobacteria, planctomyces, chlamydiae, radioresistant micrococci, and thermotogales.

6. The nucleic acid molecule of claim 4, wherein the eubacteria is selected from the group consisting of *Staphylococcus*, *Bacillus*, *Salmonella*, *Enterobacter*, *Pseudomonas*, *Arthrobacter*, *Clavibacter* and *Thermotoga*.

7. An isolated nucleic acid molecule encoding a thermostable β -glucuronidase, wherein the β -glucuronidase has a half-life of at least 10 min at 65°C.
 8. The nucleic acid molecule of claim 11, wherein the thermostable β -glucuronidase is from *Thermotoga* or *Staphylococcus* groups.
 9. An isolated nucleic acid molecule encoding a microbial β -glucuronidase, wherein the β -glucuronidase converts at least 50 nmoles of p-nitrophenyl-glucuronide to p-nitrophenyl per minute per μ g of protein at 37°C.
 10. An isolated nucleic acid molecule encoding a microbial β -glucuronidase, wherein the β -glucuronidase retains at least 80% of its activity in 10 mM glucuronic acid.
 11. An isolated nucleic acid molecule encoding a fusion protein of a microbial β -glucuronidase or an enzymatically active portion thereof and a second protein.
 12. The nucleic acid molecule of claim 11, wherein the second protein is an antibody or fragment thereof that binds antigen.
 13. An expression vector, comprising a nucleic acid sequence encoding a microbial β -glucuronidase in operative linkage with a heterologous promoter, provided that the microbial β -glucuronidase is not *E. coli* β -glucuronidase.
 14. The expression vector of claim 13, wherein the heterologous promoter is a promoter selected from the group consisting of a developmental type-specific promoter, a tissue type-specific promoter, a cell type-specific promoter and an inducible promoter.
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15. The expression vector of claim 13, wherein the promoter is functional in a cell selected from the group consisting of a plant cell, a bacterial cell, an animal cell and a fungal cell.

16. The expression vector of claim 13, wherein the vector is a binary *Agrobacterium tumefaciens* plasmid vector.

17. The expression vector of claim 13, further comprising a nucleic acid sequence encoding a product of a gene of interest or portion thereof.

18. The expression vector of claim 17, wherein the product is a protein.

19. The expression vector of claim 13, further comprising a nucleic acid sequence encoding a protein that specifically binds a cell, wherein the protein is fused to the sequence encoding β -glucuronidase and wherein the vector encodes a fusion protein.

20. The expression vector of claim 13, wherein the microbial β -glucuronidase is encoded by a nucleic acid molecule comprising nucleotides 1-1689 of Figures 4I-J or by a nucleic acid molecule that hybridizes under stringent conditions to the complement of nucleotides 1-1689 of Figure 4I-J and which encodes a functional β -glucuronidase.

21. The expression vector of claim 13, wherein the microbial β -glucuronidase comprises the amino acid sequences of Figure 5B, or a variants thereof, and which encodes a functional β -glucuronidase.

22. The expression vector of claim 13, wherein the microbe is a eubacteria.

23. The expression vector of claim 22, wherein the eubacteria is selected from the group consisting of purple bacteria, gram(+) bacteria, cyanobacteria, spirochaetes,

green sulphur bacteria, bacteroides and flavobacteria, planctomyces, chlamydiae, radioresistant micrococci, and thermotogales.

24. The expression vector of claim 22, wherein the eubacteria is selected from the group consisting of *Staphylococcus*, *Salmonella*, *Bacillus*, *Enterobacter*, *Pseudomonas*, *Arthrobacter*, *Clavibacter* and *Thermotoga*.

25. The expression vector of claim 13, wherein the microbial β -glucuronidase is a thermostable β -glucuronidase, wherein the β -glucuronidase has a half-life of at least 10 min at 65°C.

26. The expression vector of claim 25, wherein the thermostable β -glucuronidase is from *Thermotoga* or *Staphylococcus* groups.

27. The expression vector of claim 13, wherein the microbial β -glucuronidase converts at least 50 nmoles of p-nitrophenyl-glucuronide to p-nitrophenyl per minute per μ g of protein at 37°C.

28. The expression vector of claim 13, wherein the microbial β -glucuronidase retains at least 80% of its activity in 10 mM glucuronic acid.

29. The expression vector of claim 13, wherein the microbial β -glucuronidase is an enzymatically active portion thereof.

30. A host cell containing the vector according to claim 13.

31. The host cell of claim 30, wherein the host cell is selected from the group consisting of a plant cell, an insect cell, a fungal cell, an animal cell and a bacterial cell.

32. An isolated form of recombinant microbial β -glucuronidase, provided that the microbial β -glucuronidase is not *E. coli* β -glucuronidase.

33. The β -glucuronidase of claim 32, wherein the microbe is a eubacteria.

34. The β -glucuronidase of claim 33, wherein the eubacteria is selected from the group consisting of purple bacteria, gram(+) bacteria, cyanobacteria, spirochaetes, green sulphur bacteria, bacteroides and flavobacteria, planctomyces, chlamydiae, radioresistant micrococci, and thermotogales.

35. The β -glucuronidase of claim 33, wherein the eubacteria is selected from the group consisting of *Staphylococcus* group, *Salmonella* group, *Enterobacter* group, *Pseudomonas* group, *Arthrobacter* group, *Clavibacter* group and *Thermotoga* group.

36. The β -glucuronidase of claim 32, wherein the β -glucuronidase is encoded by a nucleic acid molecule comprising nucleotides 1-1689 of Figure 4I-J or by a nucleic acid molecule that hybridizes under stringent conditions to the complement of nucleotides 1-1689 of Figure 4I-J and which encodes a functional β -glucuronidase.

37. The β -glucuronidase of claim 32, comprising the amino acid sequences of Figure 5B, or a variant thereof, and which encodes a functional β -glucuronidase.

38. A method for monitoring expression of a gene of interest or a portion thereof in a host cell, comprising:

(a) introducing into the host cell a vector construct, the vector construct comprising a nucleic acid molecule according to claim 1 and a nucleic acid molecule encoding a product of the gene of interest or a portion thereof;

(b) detecting the presence of the microbial β -glucuronidase, thereby monitoring expression of the gene of interest.

39. A method for transforming a host cell with a gene of interest or portion thereof, comprising:

(a) introducing into the host cell a vector construct, the vector construct comprising a nucleic acid sequence encoding a microbial β -glucuronidase, provided that the microbial β -glucuronidase is not *E. coli* β -glucuronidase, and a nucleic acid sequence encoding a product of the gene of interest or a portion thereof, such that the vector construct integrates into the genome of the host cell;

(b) detecting the presence of the microbial β -glucuronidase, thereby establishing that the host cell is transformed.

40. A method for positive selection for a transformed cell, comprising:

(a) introducing into a host cell a vector construct, the vector construct comprising nucleic acid sequence encoding a microbial β -glucuronidase, provided that the microbial β -glucuronidase is not *E. coli* β -glucuronidase;

(b) exposing the host cell to the sample comprising a glucuronide, wherein the glucuronide is cleaved by the β -glucuronidase, such that the compound is released, wherein the compound is required for cell growth.

41. The method of claim 40, further comprising introducing into the host cell a vector construct comprising a nucleic acid sequence encoding a microbial glucuronide permease.

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42. The method of any one of claims 38-40, wherein the host cell is selected from the group consisting of a plant cell, an animal cell, an insect cell, a fungal cell and a bacterial cell.

43. A method of producing a transgenic plant that expresses a microbial β -glucuronidase, comprising:

(a) introducing an expression vector comprising a nucleic acid sequence encoding a microbial β -glucuronidase in operative linkage with a heterologous promoter,

provided that the microbial β -glucuronidase is not *E. coli* β -glucuronidase, into an embryogenic plant cell; and

(b) producing a plant from the embryogenic plant cell, wherein the plant expresses the β -glucuronidase.

44. The method of claim 43, wherein the transgenic plant is rice.

45. A method for positive selection for a transformed cell, comprising:

(a) introducing into a host cell a vector construct, the vector construct comprising nucleic acid sequence encoding a microbial β -glucuronidase, provided that the microbial β -glucuronidase is not *E. coli* β -glucuronidase;

(b) exposing the host cell to the sample comprising a glucuronide, wherein the glucuronide is cleaved by the β -glucuronidase, such that the compound is released, wherein the compound is required for cell growth

46. A transgenic plant cell comprising an expression vector, comprising a nucleic acid sequence encoding a microbial β -glucuronidase in operative linkage with a heterologous promoter, provided that the microbial β -glucuronidase is not *E. coli* β -glucuronidase.

47. A transgenic plant comprising an expression vector, comprising a nucleic acid sequence encoding a microbial β -glucuronidase in operative linkage with a heterologous promoter, provided that the microbial β -glucuronidase is not *E. coli* β -glucuronidase.

48. A seed from the transgenic plant of claim 47.

49. A transgenic aquatic animal cell comprising an expression vector, comprising a nucleic acid sequence encoding a microbial β -glucuronidase in operative linkage with a heterologous promoter.

50. A transgenic aquatic animal comprising an expression vector, comprising a nucleic acid sequence encoding a microbial β -glucuronidase in operative linkage with a heterologous promoter.

51. A method for identifying a microorganism that secretes β -glucuronidase, comprising:

(a) culturing the microorganism in a medium containing a substrate for β -glucuronidase, wherein the cleaved substrate is detectable, and wherein the microorganism is an isolate of a naturally occurring microorganism or a transgenic microorganism; and

(b) detecting the cleaved substrate in the medium;
therefrom identifying an organism that secretes β -glucuronidase.

52. The method of claim 51, wherein the microorganism is isolated from soil, mud, skin, mucus or fecal matter.

53. The method of claim 51, wherein the microorganism is cultured under conditions unfavorable to growth of *Staphylococcus* and favourable to other microorganisms.

54. A method for providing an effector compound to a cell in a transgenic plant, comprising:

(a) growing a transgenic plant that comprises an expression vector, comprising a nucleic acid sequence encoding a microbial β -glucuronidase in operative linkage with a heterologous promoter and a nucleic acid sequence comprising a gene encoding a cell surface receptor for an effector compound.

(b) exposing the transgenic plant to a glucuronide, wherein the glucuronide is cleaved by the β -glucuronidase, such that the effector compound is released.

55. The method of claim 54, further comprising introducing into the transgenic plant a vector construct comprising a nucleic acid molecule encoding a glucuronide permease.

56. The method of claim 55, further comprising introducing into the transgenic plant a vector construct comprising a nucleic acid sequence that binds the effector compound.

57. The method of claim 56, further comprising a gene of interest in operative linkage with the nucleic acid sequence that binds the effector compound.

58. The method of claim 54, wherein the effector compound is hydrophobic.

59. The method of claim 56, wherein the effector compound is either ecdysone or a glucocorticoid.